AN ADDRESS

ON

CHOLERA AND ITS BACILLUS.

Delivered before the Imperial German Board of Health, at Berlin.

BY ROBERT KOCH, M.D.[1]

[BEFORE the opening of the sitting, some microscopic preparations were exhibited by Dr. Koch, which will be referred to afterwards. Dr. Koch also explained the method for preparing and carrying out the cultivation of the cholera-bacillus.

The preparation takes place in the usual manner. A drop of mucus from the dejecta, or from the contents of the intestines, is spread on an object-glass, and dried. The object-glass is then drawn three times through the flame of a gas- or spirit-lamp, sprinkled with a watery solution of fuchsine or methyl-blue, and rinsed after a few seconds, in order to be immediately investigated under the microscope with the help of an oil-system, one-twelfth inch, and Abbé's condenser.

Sections from the intestine, which must be hardened in pure alcohol, are best coloured in a strong watery solution of methylene-blue for twenty-four hours, or by being warmed for a short time, and then treated in the usual manner.

The microscopic test alone is sufficient in only a comparatively few cases for the diagnosis, and generally the following method of cultivation is necessary for proving the certain presence of the comma-bacillus.

A very small drop of mucus is placed in 10 cubic centimètres of food-gelatine (meat-water-pepton-gelatine, containing 10 per cent. of gelatine, and having a weak alkaline reaction), and distributed in it by putting the fluid in motion. Then the liquid gelatine is poured on to a glass plate lying horizontally, which is cooled by means of ice lying under it. The gelatine, spread out by a sterilised glass-rod, very speedily congeals. The plate is then put under a glass-receiver, which is kept damp till the colonies of bacteria develop, and is then examined with a Zeis A. A. eye-piece 4, or a corresponding power.]

Gentlemen,—For sanitary measures, we require bases of as firm a foundation as possible. It is not only a question of very costly institutions, but of the happiness and misery of many people. This is most especially true for protection from pestilences, in which, it can be said without exaggeration, the most important sanitary efforts are being engaged. We should therefore suppose that, in the struggle against pestilences, people would start from thoroughly established and scientifically elaborated bases; but unhappily this is not everywhere the case, and especially with regard to cholera such a firm basis is wanting. It is true that a host of views on the nature of cholera, and its mode of speeading and infection, have been expressed, and various theories have been propounded concerning it; but the opinions are still so very divergent, they are so diametrically opposed, that we cannot take them, without examination, as supports or starting points for the measures we want to take in combating this plague.

It is asserted, on the one hand, that the cholera is a specific disease originating in India; on the other hand, this is disputed, and it is held that cholera can also arise spontaneously in other countries, and is not dependent on a specific cause. Some hold that cholera is only introduced by the cholera-patient and his effects; others say that it can be spread by merchandise, by people in good health, and by currents of air. Equally contradictory opinions exist concerning the importance of drinking-water as a vehicle for conveying the infectious matter, and concerning the influence of the conditions of the soil, and concerning the question whether or not the infectious matter is contained in the dejecta of the patient, and on the duration of incubation. But all these are precisely points of the greatest importance for the protection against cholera, and a successful resistance to the disease will not be possible till some unity of opinion has been arrived at on these fundamental questions of the etiology of cholera.

The etiology of cholera has profited little from the progress which we have made in the knowledge of the etiology of other infectious diseases. This progress has developed chiefly in the last ten years; and during this period there has been no opportunity of investigating cholera—at least, not in Europe or in adjacent countries; and in India, where the cholera could have continuously afforded material for investigation, nobody has been found to occupy himself with this task by applying the new methods of investigation.

Therefore, in this respect, it was not unfavourable that the cholera broke out last year in Egypt, and that opportunity was thus given for studying the nature and mode of infection of this disease before it arrived on European soil. This opportunity was utilised by various Governments, which sent out expeditions to investigate the nature of cholera. I had the honour of superintending one of these expeditions.

When I undertook this commission, I was fully aware of the difficulties of the task. Properly speaking, nothing was as yet known of the infectious matter of cholera. It was not known where to look for it—whether it existed in the intestinal canal, or in the blood, or elsewhere. It was, further, not known whether one had to deal in this case also with bacteria, or with fungi or something similar, or with animal parasites—*e. g.*, amœbn. It is true that, in this respect, there were not such important difficulties met with as in another direction, where I least expected to find them. I had pictured to myself the pathological appearance, constructed according to the description of the ordinary text-books; and had supposed that the intestine in cholera showed very few modifications, and that it was filled with a fluid resembling rice-water. The sections that I had previously seen I had already half forgotten, so that I could not correct this false idea. Hence I was at first rather surprised and uncertain when I came to see something else in the intestine. In the first sections, it was at once evident that, in by far the greater majority of cases, extremely great and striking modifications were to be found. Other cases, again, showed slight modifications; and, finally, I came across cases which in some degree corresponded to the type given in the ordinary books of instruction. But I had to wait some time, and make several sections, before I succeeded in obtaining a correct conception, and in explaining all these various modifications that I had come across.

I will at once remark here that, in spite of the most careful investigation of all the other organs and of the blood, nothing was to be found which could lead one to suppose that the infectious material was to be found there. My attention, therefore, was concentrated exclusively on the modifications in the intestines, and these may be grouped in the following manner.

There were cases in which the lower section of the small intestine was coloured dark brownish red, most intensely immediately above the ileo-cæcal valve, less so higher up, the mucous membrane being studded with superficial hæmorrhages. In many cases the mucous membrane was even superficially necrotised and provided with diphtheritic coatings. Corresponding to this, the contents of the intestine were not a rice-watery colourless fluid, but a bloody, ichorous, stinking fluid.

Other cases showed a gradual transition to less marked modifications. The redness was less intense in them, and finally existed only in patches; and these were followed by cases in which only the borders of the follicles and Peyer's glands were reddened. This last mentioned form affords a very characteristic appearance, which does not occur in other affections of the intestines, and is quite peculiar to cholera. In comparatively very few cases, however, the mucous membrane was strikingly little changed. It looked somewhat swollen and less transparent in the surface-layers; the solitary follicles and Peyer's glands were more strongly prominent. The whole mucous membrane was slightly rose-coloured, but there was nowhere any capillary hæmorrhage. In these cases the contents of the intestine also looked colourless, but they were by no means always like rice-water, but would be generally better compared to gruel. Only in a few cases have I seen that the contents of the intestine were purely watery and mucous, and contained comparatively few flakes.

When we examined the intestine and its contents under the microscope, it was seen that, in some cases, especially in those in which the Peyer's glands were red at the edge, an invasion of bacteria corresponding to this redness had taken place. The following diagram illustrates the appearance as you have already seen it in one of the preparations I showed you. The bacteria had partly forced their way into the utricular glands, partly pushed themselves between the epithelium and the basement-membrane, thereby lifting the epithelium as it were. In other parts, it was seen that they had forced their way deeper into the tissue. Then cases were found in which, behind these bacteria, which had a special appearance with regard to size and shape, so that one could distinguish them from other bacteria, and devote special attention to them, various other bacteria forced their way into the utricular glands and the surrounding tissue, *e.g.*, large thick bacilli and very thin bacilli. Thereby conditions are produced similar to those in necrotic diphtheritic changes of the mucous membrane of the intestine, and in typhoid ulcers, where afterwards other non-pathogenic bacteria force their way into the tissue rendered necrotic by pathogenic bacteria. We were, therefore, from the very beginning, obliged to look upon these first mentioned bacteria as not altogether unimportant for the cholera-

[1] Translated from the *Deutsche Medicinische Wochenschrift* for the BRITISH MEDICAL JOURNAL.

process, whilst everything else gave the impression that it was something secondary; for the bacteria first described always advanced beyond the others, they forced their way farther in, and gave the impression as if they had smoothed the way for the other bacilli.

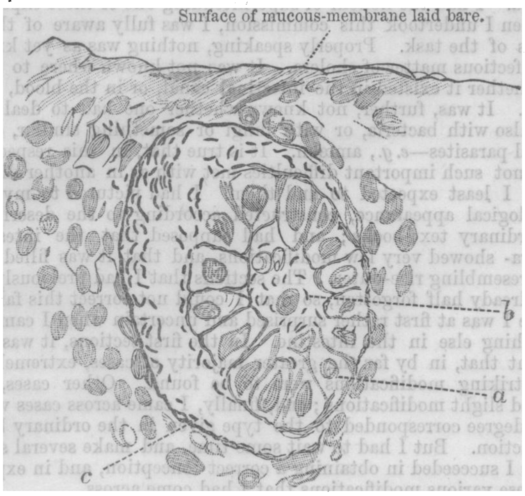


Fig. 1.—Section of the Mucous Membrane of a Cholera-intestine. An utricular gland (*a*) is diagonally cut through. In its interior (*b*), and between the epithelium and basement-membrane (*c*), numerous comma-bacilli. 600 magnifying power.

With regard to the contents of the intestine, at first no clear idea could be formed, as the only cases which came before us for examination were not suitable; in these, also, the contents of the intestine were already putrid and bloody. There were an enormous quantity of various bacteria in these contents, so that there was no possibility of attending to the real cholera-bacilli. Not till I had dissected a couple of acute and uncomplicated cases, in which no hæmorrhage had as yet set in, and in which the contents of the intestines had not yet turned to putrid decomposition, did I recognise that, the purer and fresher the cases, the more did a special kind of bacteria prevail in the contents of the intestines also, and it was soon clear that these were the same bacteria which I had seen in the mucous membrane. This discovery naturally turned my attention more and more to this kind of bacteria. I investigated them in all kinds of ways, in order to establish their special peculiarities; and am able to give the following information regarding them.

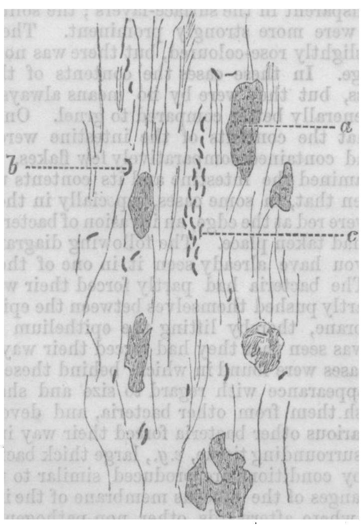


Fig. 2.—Object-glass preparation from the contents of a Cholera-intestine. Core of the Necrotised Epithelia (*a*). Semicircular Comma-bacillus (*b*). Specially characteristic grouping of Comma-bacilli (*c*). 600 magnifying power.

These bacteria, which I have called comma-bacilli, on account of their peculiar shape, are smaller than the tubercle-bacilli. One scarcely forms a correct idea of the thickness, length, and breadth of bacteria by giving their dimensions in numbers; I therefore prefer to compare the dimensions of bacteria with other objects, so that one can immediately form a tolerably good idea. As the tubercle-bacilli are known to everybody, I will compare the cholera-bacteria with them. The cholera-bacilli are about half, or at most two-thirds, as long as tubercle-bacilli, but much more bulky, thicker, and slightly curved. This curve is generally not more marked than that of a comma; but sometimes it is larger, becoming semicircular, as in the adjoining figures (Figs. 2 and 3). In other cases, it is seen that the curve is doubled, that one comma is attached to another, but in an opposite direction, so that it forms the shape of S. I think that, in both cases, two individual ones after being divided have remained stuck together, and accordingly give the appearance of a more marked curve. But in the artificial cultivations, besides these, another very remarkable form of development of the comma-bacillus is to be found, which is very characteristic of it.

The comma-bacilli frequently grow in threads of longer or shorter length. In one of the preparations I have laid before you (shown in the following diagram) several examples of this form can be seen (fig. 4).

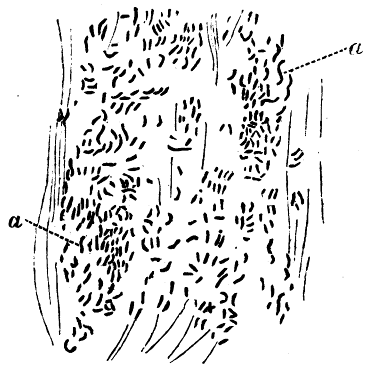


Fig. 3.—Object-glass preparation. Cholera-dejecta on Damp Linen (two days old). Great multiplication of Comma-bacilli, amongst which some S-shaped (*a*). 600 magnifying power.

But they do not then form straight threads, like other bacilli, for instance, anthrax-bacilli, or, as it appears in the microscopic picture, simple wavy threads, but very tender long spirals, which, as far as their length and the rest of their appearance are concerned, bear the closest resemblance to the spirochætæ of relapsing fever. I could not distinguish one from another if I had them side by side. Owing to this peculiar form of development, I am also inclined to the view that the comma-bacillus is not a genuine bacillus, but that it is, properly speaking, a transition-form between bacilli and spirilla. Perhaps, indeed, we have here to deal with a genuine spirillum, of which we have a fragment before us. It is seen, also, in other spirilla—for instance, in spirilla undula—that very short specimens do not form the complete thread of a screw, but only consist of a short little staff, which is more or less curved. I shall return later to this point, which is by no means devoid of importance.

The comma-bacilli can be cultivated in meat-broth. They grow in this liquid extremely quickly, and in great numbers; and this property of theirs can be utilised for studying their other qualities, by examining, with a strong magnifying power, a small drop of a meat-broth cultivation on the object-glass. It is then seen that the comma-bacilli move in a very lively manner. When they are collected together at the edge of the drop, and are moving about amongst one another, they look like a swarm of dancing midges, and those long spiral threads appear also moving in an animated manner, so that the whole affords a strange and extremely characteristic picture.

But the comma-bacilli also grow in other liquids, and especially, speedily and in great abundance, in milk. They do not make milk curdle, and do not precipitate the casein, which many other bacteria, which can also be raised in milk, do. Hence the milk looks quite unchanged; but if you take a small drop from the surface, and examine it under the microscope, it teems with comma-bacilli. They also grow in the serum of blood, in which they also very quickly develop, and multiply in great numbers. A very good soil for the reproduction of comma-bacilli is also food-gelatine. This gelatine can serve for facilitating and securing the discovery of comma-bacilli; for the colonies of comma-bacilli assume, in the gelatine, a most characteristic and definite form, which, so far as I can discern, and as far as my experience reaches, no other kind of bacteria assumes in like manner.

The colony looks, when it is very young, like a very pale and tiny little drop (Fig. 4), which is, however, not quite circular, the shape

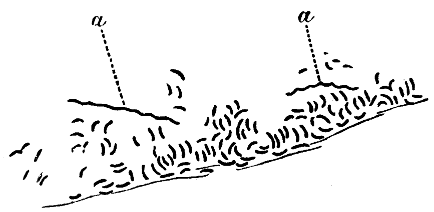


Fig. 4.—Object-glass preparation. From the edge of a drop of Meat-broth, with pure-culture of Comma-bacilli. Long screw-shaped Threads (*a*). 600 magnifying power.

generally assumed by these bacteria-colonies in gelatine ; but it has a more or less irregularly bordered, hollowed out, in parts also rough or jagged, shape. It also has, at a very early stage, rather a granulated appearance, and is not of such regular character as other colonies of bacteria.

When the colony becomes somewhat larger, this granulation becomes more and more evident ; at last it looks like a little heap of strongly refracting granules. I might best compare the appearance of such a colony with the appearance of a little heap of pieces of glass. As they grow, the gelatine liquefies in the immediate neighbourhood of the bacteria-colony, and this latter sinks down at the same time deeper into the mass of gelatine. A funnel-shaped cavity is thus formed in the gelatine, in the midst of which the colony is seen as a little whitish point (as seen in the diagram). This appearance is also quite peculiar ; it is seen, at least in this manner, in very few other kinds of bacteria, and, as far as I know, never so marked as with the comma-bacilli. The sinking of the colonies can be best observed when carrying out an artificial cultivation. A suitable colony is selected on the gelatine-plate, under a microscope with a glass of slightly magnifying power ; it is touched with a platinum-wire, previously heated ; the bacilli are transferred by the wire into a test-tube with gelatine, and this is closed with sterilised wadding. A cultivation of this kind then grows in the same manner as the colony on the gelatine-plate. I am in possession of a numerous collection of artificial cultivations of bacteria, made in this manner ; but I have never seen in their case such changes as the comma-bacilli cause after being transferred into the gelatine. Here, also, as soon as the cultivation begins to develop, you see a little funnel, which marks the point where the inoculation took place (see the diagram). By degrees, the gelatine liquefies in the neighbourhood of this point of inoculation ; then the little colony is plainly seen, extending itself more and more; but a deep spot, sunken in, always remains, which looks, in the partially liquefied gelatine, as if an air-bubble were hovering over the colony of bacilli. It almost gives the impression as if the bacilli-vegetation not only caused a liquefaction of the gelatine, but also a speedy evaporation of the liquid formed. We already know a number of other kinds of bacteria which, in quite the same manner, gradually liquefy the gelatine in test-tubes, starting from the point of inoculation. But in these cases, there is never such a cavity, nor this bubble-like hollow space. I must still mention that the liquefaction of the gelatine, starting from a single isolated colony, the best way of observing it in a layer of gelatine, which is spread out on the glass-plate, never spreads very wide. The dimension of the liquefied district of a colony may be estimated at one millimètre. Other kinds of bacteria can liquefy the gelatine to a much greater extent, so that a colony attains a size of one centimètre in diameter, and more. In the cultivations of comma-bacilli, made in test-tubes, the liquefaction of the gelatine extends by degrees and very slowly, starting from the point of inoculation ; and continues in such a manner that, after about a week, the whole contents of the tube have become liquid. Unimportant as all these qualities seem in themselves, special weight is to be laid on them, because they serve to distinguish comma-bacilli from other kinds of bacteria.

Comma-bacilli can also be cultivated on Ceylon moss *(Agar-agar)*, to which meat-broth and pepton are added. This agar-agar jelly is not liquified by the comma-bacilli. They can also be raised on boiled potatoes—a fact which is very important for certain questions. They grow on potatoes exactly like the bacilli of glanders. The latter form, as was seen at the Hygienic Exhibition last year at Berlin, a thin, pulpy, brownish coating on the potatoes. The cultivations of comma-bacilli, when grown on potatoes, look like this, but not coloured so intensely brown, but rather light greyish brown.

Comma-bacilli flourish best at temperatures between 30° and 40° Cent. (86° to 104° Fahr.), but they are not very susceptible to lower temperatures. Experiments have been made on this point, which show that they can grow very well at 17° Cent., though more slowly. Below 17° Cent., the growth is very small, and seems to cease below 16°. In this point, the comma-bacilli remarkably resemble anthrax-bacilli, which also have this minimum temperature as the limit for their growth-power. Once I made an experiment to test the influence of lower temperatures on comma-bacilli, and to see if they are not, at a very low temperature, not only hindered in their development, but also if they cannot possibly be killed. For this purpose, an artificial cultivation was exposed for an hour to a temperature of 10° Cent. below zero ; during this time, it was completely frozen. When part of it was put into the gelatine, there was not the least difference visible in the development or growth so that they bear frost very well. It is not the same with the withdrawal of air and oxygen. They immediately cease to grow when deprived of air, and accordingly belong, if the division into aërobic and anaërobic bacteria be held as good, to the aërobic class. Anyone can convince himself of this very simply, by laying a piece of talc or mica over the glass plate, when the portion of the artificial cultivation has been placed on it in liquid gelatine, and when the gelatine is beginning to stiffen ; the talc or mica must be as thin as possible, and must cover at least one-third of the gelatine surface in the middle. The piece of mica, owing to its elasticity, adheres completely to the surface of the gelatine, and thus cuts off the air on the portion covered. Then, as soon as the development of the colonies follows, it is seen that the development only takes place where the gelatine is not covered, and only a trifle, about two millimètres, under the mica-plate, up to which point the air has been able to force its way. But under the mica-plate itself nothing grows. Extremely small colonies, invisible to the naked eye, do, it is true, appear, which probably owe their origin to the oxygen existing in the gelatine, but they do not increase in size afterwards. An experiment was made in another manner. Little glasses containing food-gelatine, which had been inoculated with comma-bacilli, were placed under an air-pump, and others prepared in the same manner were kept outside the air-pump. It was then seen that those under the air-pump did not grow, but only those outside it. But when those that had been under the air-pump were again placed in the air, they began to grow. Hence they had not died ; they only wanted the necessary oxygen to be able to grow. The same occurs when the cultivations are brought into an atmosphere of carbonic acid. Whilst the cultivations that have been kept for comparison outside the carbonic acid atmosphere grow in the usual manner, those that are in a stream of carbonic acid remain undeveloped. But in this case, also, they do not die ; for, after having been for some time in the carbonic acid, they begin to grow immediately after they have come out of it.

On the whole, comma-bacilli, as I have repeatedly observed, grow extremely rapidly. Their vegetation very speedily reaches a maximum, at which it only remains stationary for a short time, then diminishing again very speedily. The comma-bacilli, when wasting away, lose their shape ; they appear at one time shrivelled, and at another time swollen, and in this state they are not at all, or only slightly, susceptible to colour. The peculiar conditions of vegetation of comma-bacilli can be best observed by bringing substances which are rich in comma-bacilli, but also contain other bacteria, *e.g.*, the contents of a cholera-intestine or cholera-dejecta, in contact with moist earth, or by spreading them out on linen, and keeping them in a damp condition. Comma-bacilli then increase in a very short time, *e.g.*, in an extraordinary manner in twenty-four hours. Other bacteria that exist with them are at first stifled by the comma-bacilli, a natural pure culture is formed, and, on examining with the microscope the mass that is taken from the surface of the damp earth or linen, preparations can be obtained which show almost exclusively comma-bacilli. Such a preparation is the following, that comes from the damp linen of a cholera-patient polluted with his dejecta. (Fig. 5.)

But this luxuriant growth of comma-bacilli does not last long. After two or three days, they begin to die off, and other bacteria then increase. The conditions become the same as in the intestine itself. There also a rapid multiplication takes place ; but when the real vegetation-period, which only lasts for a short time, is over, and especially when exudations of blood into the intestine take place, the comma-bacilli disappear, and the other bacteria, especially putrefaction-bacteria, commence to develop in their room. I am, therefore, almost inclined to believe that, if the comma-bacilli were brought at first into a putrefied liquid which contained a great deal of the products of vital changes of other bacteria, and especially of putrefaction-bacteria, they would not come to development, bnt would soon die off. But, so far, sufficient experiments have not been made on this point; that is only a supposition which I make, supported by my experiences of other bacteria-cultivations. This point is important, because it is not a matter of indifference whether the comma-bacilli, if they come into a sink, find a good or a very bad soil for reproduction. In the first case, they would multiply, and would have to be destroyed by methods of disinfection; but in the latter case they would die off, and there would be no necessity for disinfecting. I am inclined to hold the latter view, as borne out by all the experience I have so far had.

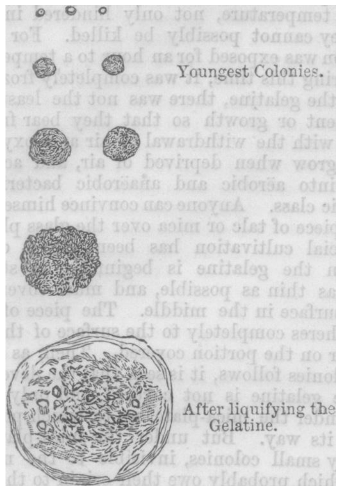
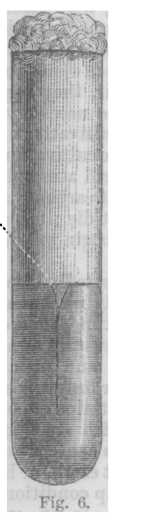


Fig. 5.—Colonies of Comma-bacilli on the Gelatine-Plate. 80 magnifying power.



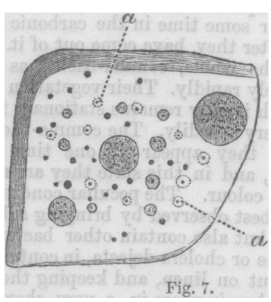


Fig. 6.—Funnel-shaped Sinking in the Gelatine at the Inoculation-point in the Test-tube.
Fig. 7.—Natural Size of the Colonies as they appear on the Gelatine-Plate.

The comma-bacilli flourish best in liquids which do not contain too small a quantity of nutritive matters. Several experiments have been made on this point. Dilutions of meat-broth with an alkaline reaction were prepared, and a quantity of comma-bacilli were placed in them. In one of these experiments, the meat-broth, after a fivefold dilution, proved to be no longer a nutritive solution. In other experiments, the bacilli grew in a tenfold dilution. Of course, these experiments must be repeated, and be made in a more extensive manner, in order to find a definite and fixed limit; but, in any case, it can be seen from these results that one must not dilute too much, and that comma-bacilli require a certain concentration of nutritive substance in which to grow.

In these cnltivation-experiments, it was further seen that the nutritive substances—at least, the gelatine and meat-broth—must not be acid. As soon as the gelatine shows only a trace of acid reaction, the growth of the comma-bacilli is very stunted. If the reaction be in a marked degree acid, the development of the bacilli completely ceases. It is at the same time noteworthy that it is not all acids that seem to be unfavourable to the comma-bacillus; for the surface of a boiled potato, where it is cut, is known to have an acid reaction, in consequence, if I am not mistaken, of its containing malic acid. Nevertheless, comma-bacilli grow very luxuriantly on potatoes. Hence, one cannot say, straight off, that all acids hinder the growth; but, in any case, there are a number of acids which have this effect. In meat-broth it is probably lactic acid, or an acid phosphate.

As the influence of substances that prevent the development of the growth of comma-bacilli is one of no small interest, a number of other substances have been examined with regard to this point. I must observe here that the prevention of development does not imply disinfection. In these experiments, it is only intended to determine that amount of a substance which is sufficient to hinder the growth of the bacteria. But with this the bacteria are by no means killed outright, as should be done in disinfection. We had experienced something similar in the experiments on the influence of carbonic acid on comma-bacilli, in which also the growth was restrained only as long as the carbonic acid was allowed to operate. The same holds for the statements which I now lay before you.

Iodine is known to have been characterised by Davaine as a very intense poison for bacteria, and, under certain circumstances, correctly so. Davaine made his experiments by diluting, to a very great extent, a liquid containing anthrax-bacilli, *e.g.*, anthrax-blood, to such an extent, that he finally had nothing but pure water, in which very few anthrax-bacilli were suspended. He added some iodine to this liquid, and then it was seen that the anthrax-bacilli were killed by an extremely small quantity of iodine; but in practice the conditions are quite the reverse. We never have to stop the development of infectious matters in pure water, but in the alkaline contents of the intestines, or in the blood or in the juices of the tissues, and the iodine does not remain free in these, but combines at once with the alkalies. The investigation of the influence of iodine on the comma-bacilli was made by adding iodine-water to meat-broth, which was just suitable as a nutritive liquid. Iodine dissolves in water in the proportion of about 1 in 4,000. One cubic centimètre of this iodine-water was mixed with ten cubic centimètres of meat-broth, but this did not hinder the growth of the bacilli in the least; the limit at which iodine prevents the bacilli from developing must, therefore, lie far below the amount used in this experiment. But it seems to me unnecessary to make any more experiments on this point, as, in practice, larger quantities of iodine than this cannot be given.

Alcohol stops the development of comma-bacilli only when one part is added to ten parts of a nutritive fluid, *i.e.*, in the proportion of ten per cent. This is a concentration which also cannot be practically utilised.

Common salt was used in the proportion of 2 per cent. without the growth of the comma-bacilli being hindered.

Sulphate of iron only hinders the growth when two per cent. is added to the nutritive fluid. In regard to this substance, which is very much used for purposes of disinfection in times of cholera, I venture to remind you that a proportion of two per cent is necessary before it acts as a preventive to development. The comma-bacilli are not yet killed by the sulphate of iron in this concentration. The property which sulphate of iron has, of hindering the development of the bacilli is, perhaps, thus explained. The peptone and albuminates of the nutritive solution, which serves as food for the bacteria, are driven out; for, by adding two per cent. of sulphate of iron, an abundant precipitate is formed in the nutritive solution. Possibly, also, the acid reaction that takes place has a checking effect on the growth. Accordingly, this substance seems not to possess any specific effect on the bacteria, and certainly not to be a real material for killing or disinfection. I consider it indeed possible that, with such a substance, exactly the opposite of what is intended may be obtained. Given the case that the contents of a cesspool had to be disinfected, into which it was known that comma-bacilli had found their way; according to my view, the process of putrefaction that goes on of itself in the cesspool is sufficient to kill the comma-bacilli. But if sulphate of iron be added till there be an acid reaction, and the process of putrefaction is thereby arrested, nothing else is obtained but cessation of the growth of the bacteria and of the comma-bacilli. The bacteria are by no means killed by this method; and, as for the comma-bacilli, they are removed from the influence of the putrefaction-bacteria which are injurious to them, and are preserved instead of being destroyed.

This example is a very good one to show that the substances for disinfection must be correctly judged and examined precisely on this point, and that we have to distinguish between what only arrests putrefaction, and what really kills bacteria. The former may very possibly serve as a means of preserving infectious matters.

I will only mention the limit of the power other substances possess of arresting the development: alum, 1 : 100 ; camphor, 1 : 300. I had expected a stronger effect from camphor, but several careful experiments have shown that this substance possesses only a very slight influence on comma-bacilli. Carbolic acid, 1 : 400. This figure nearly agrees with what we know of the influence of carbolic acid on other bacteria. Peppermint-oil, 1 : 2,000. Sulphate of copper, 1: 2,500. This substance has a very powerful effect. But if we want to calculate how much sulphate of copper must be given in order to check the growth of the bacilli in the intestinal canal, we should arrive at quantities which could not be given to a human being. Quinine 1 : 5,000 ; and corrosive sublimate, which is here again seen to exceed all other substances in power, 1 : 100,000.

In these experiments on the influence of substances for arresting the development of comma-bacilli, the striking fact was evident that comma-bacilli die off extremely easily when dried. These experiments were made by letting a very small drop of a substance containing bacilli dry on an object-glass, and a large supply of these object-glasses were immediately prepared for a series of experiments. A drop of the liquid which was to be examined was then placed upon such an object-glass, and left for development in the hollow object-holder. Having proceeded in this manner, in no single preparation did anything grow that had received meat-broth as nutritive fluid, nor in a striking manner in the test-preparations either. At first I did not know what caused the absence of growth, and thought that the broth must be the cause of it, for I had never met with anything like this before in the case of other bacteria. For instance, anthrax-bacilli can be kept in store for a long time dry on object-glasses ; they retain vitality from half a week to nearly a whole week in this manner. As, however, the meat-broth on examination proved to be unexceptionable, we had to examine whether the comma-bacilli had not probably died off owing to being dried upon the object-glass. In order to obtain certainty on this point, the following experiment was made. A number of object-glasses were provided with a small drop of substance containing bacilli. The drop dried up in a few minutes. One object-glass was now diluted with a drop of meat-broth after an interval of a quarter of an hour, another after an interval of half an hour, another after an interval of an hour, and so on. Then it was seen (and I made several series of experiments) that the comma-bacilli did come to development on the dried glass-plates that had laid a quarter, a half, and a whole hour. but after two hours they sometimes died off ; after three hours, I could not keep the bacilli alive in these experiments. Only when compact masses of bacilli-cultivations—for instance, when the pappy substance of a cultivation made on potatoes was dried—did the bacilli retain vitality for a longer time; clearly because in this case complete desiccation followed much later. But, also, under these conditions I have never succeeded in preserving the bacilli alive in a dried state longer than twenty-four hours.

This result was in so far important, as by its means it could easily be tested whether the bacteria have a permanent state. We know that other pathogenic bacteria—for example, anthrax-bacteria, which form spores, can be preserved for years in a dry state on an object-glass without their dying. We know also of other infectious substances, with whose nature we are not yet accurately acquainted for example, the infectious matters of small-pox and of vaccine, which can be kept in a dried state for several years, still retaining their power of infection. If now the comma-bacilli, which, as such, are unexceptionally speedily killed by drying, pass into a lasting condition under some circumstances, that would be very soon shown during the process of drying.

This is anyhow one of the most important questions for the etiology of an infectious disease, and especially so for cholera. The investigation of this point has therefore been made in the most careful manner possible, and in every possible manner, and I hardly think that anything more can be done on this point. Above all, cholera-dejecta and the contents of the intestines of cholera-corpses were left in a damp condition on linen, in order that the comma-bacilli might develop under the most favourable circumstances. After certain intervals of time, pieces of the linen were dried, for example, after twenty-four hours, after a few days, after several weeks, to see if, during this period, any condition of permanence had been established. For infection through cholera-linen affords the only undisputed example of the presence of an effectual infectious substance, which adheres to a special object. If there were a permanent state to be found anywhere, it must have been found on cholera-linen.

But in none of these cases was a permanent state discovered. When the dried things were examined, it was seen that the comma-bacilli had died off. Then, further, the dejecta were placed in earth, being either mixed with earth or spread on the surface, which was either kept dry or moist; they were mixed with marsh-water, and were also left to decay without anything being added to them. In gelatine-cultivations, the comma-bacilli have been cultivated up to six weeks, also in serum of blood, in milk, on potatoes, on which anthrax-bacilli are known to form spores extremely rapidly and in great abundance. But we have never obtained a permanent state of comma-bacilli. As we know that the majority of bacilli have a permanent state, this result must appear very striking. But I will remind you here, what I mentioned before, that we have most probably to deal with a micro-organism here, which is not a genuine bacillus at all, but is more allied to the group of screw-shaped bacteria, spirilla ; but we do not know of any permanent state of spirilla as yet. Spirilla are bacteria which depend for their existence exclusively on liquids, and do not, like anthrax-bacilli, vegetate under certain conditions in which they have for once to endure a dry state. It, therefore, seems to me, as far, at least, as my experience goes, that there is no prospect of finding a permanent state of comma-bacilli. I shall later on explain that the absence of a permanent state perfectly coincides with the experiences of the etiology of cholera.

[*To be continued.*]

REMARKS ON THE PROPHYLACTIC POWER OF ARSENIC IN MALARIA.

By Edward Drummond, M.D., Rome.

As to the prophylactic power of arsenic in enabling persons exposed to the infection of scarlatina, diphtheria, etc., to resist such poisons, referred to by Dr. Walford in the Journal of July 5th, I am unable to speak from experience ; but that it has such power against malaria seems to be established on more than merely *primâ facie* evidence. Such an experiment is open to so many sources of fallacy, that only repeated observations made under the strictest safeguards can settle the question.

In a suggestive report made by Dr. Tommasi-Crudeli to the Italian Minister of Agriculture in March 1883, some very important facts were adduced. The trial of arsenic in this way was proposed by him in a communication to the Accademia dei Lincei, December 1880, and was carried out by the medical staff in charge of the Roman and South Italian railways, amongst the men employed, who are largely exposed to the infection of malaria. In 1881, Dr. Ricchi, chief of the staff, instituted such experiments, but the year was one of feeble malarial activity. In 1882, the malaria was much more active, and Dr. Ricchi caused arsenic to be administered to 455 persons, so exposed, commencing with one milligramme, gradually increased to eight milligrammes for each person daily. Of these 455, 338 were cured of fevers which they had, or prevented from contracting such. In 43 cases, the results were negative; in 74, doubtful. Amongst the negative cases were included those who did not take the remedy regularly, or only for a few days. The doubtful class alternated the arsenic with other supposed prophylactics. Dr. Ricchi, therefore, continues his observations, and is convinced that, "if arsenic is not always preservative against the malarious infection, it renders the human organism less and less susceptible to the ferment of malaria." Like conclusions followed from the ase of arsenic amongst persons employed by Messrs. Piacentini, in the Roman Campagna ; by Prince Corsini, in the Tuscan Maremma, amongst the men employed in the Royal Chases at Castelparciano. The results are borne out by experiments on animals, made by Marchiafava and Cuboni, who inoculated these with the blood of malarious fever.

Further researches were instituted, under the most careful safeguards against error, by preparing animals for inoculation with malarious blood ; one-half being treated with arsenic, and the other half without. Owing, however, to the remarkable paucity of cases of pernicious fever, Professor Marchiafava, Drs. Celli, Ferrareri, and others, have not since been able to obtain the necessary supply of the blood of pernicious fever, and the experiments have not hitherto been able to be carried out in their integrity.